

Gel Purification using Qiaquick Gel purification kit (Qiagen)

1. Run the samples in a SeaKem gel.
2. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel/razor blade.
3. Weigh the gel slice in a colorless tube. Add 900 μ l of Buffer QG (no matter what mass the gel is).
4. Incubate at 42°C for 10 min (or until the gel slice has completely dissolved). To help dissolve the gel, mix by vortexing the tube every 2-3 min during the incubation.
5. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color of the mixture is orange or violet, add 10 μ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
6. Add 400 μ l of isopropanol to the sample and mix.
7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.
8. Discard flow-through and place QIAquick column back in the same collection tube.
9. Add 500 μ l of Buffer QG to QIAquick column, incubate at room temperature for 1 min, and centrifuge for 1 min. Discard flow-through.
10. Add another 500 μ l of Buffer QG to QIAquick column and centrifuge immediately for 1 min. Discard flow-through.
11. To wash, add 750 μ l of Buffer PE to QIAquick column, incubate at room temperature for 5 min, and centrifuge for 1 min. Discard flow-through.
12. Add another 500 μ l of Buffer PE to QIAquick column and centrifuge immediately for 1 min.
13. Discard flow-through and centrifuge the QIAquick column for an additional 1 min at 13,000 rpm ($\sim 17,900 \times g$).
14. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
15. To elute DNA add 50 μ l of water or 0.1X Buffer EB to the center of the QIAquick membrane, let the column stand for 5 min, and centrifuge the column for 1 min.
16. Elute again with 50 μ l of water or 0.1X Buffer EB and centrifuge immediately for 1 min.